IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.:	**BERN-0040**
Inventors:	**Eric F. Bernstein**
Serial No.:	**09/913,697**
Filing Date:	**January 28, 2002**
Examiner:	**Dodson, Shelley A.**
Group Art Unit:	**1616**
Title:	**Compositions and Methods for Prevention of Photoaging**

DECLARATION UNDER RULE § 1.131

I, Eric F. Bernstein, hereby declare that:

1. I am a co-inventor of the above-identified application and am most familiar with the subject matter of this application and the research effort occurring in my laboratory located in Philadelphia, Pennsylvania which lead to the discovery of this invention.

2. As evidenced by pages from laboratory notebooks attached hereto, experiments have been performed demonstrating the ability of serine protease inhibitors to protect against cutaneous photodamage in a transgenic mouse model containing the human elastin promoter linked to a chloramphenicol acetyltransferase (CAT) reporter gene. This model is well-accepted for its utility in testing compounds that may inhibit cutaneous photodamage in humans.

3. The experiments described in the attached laboratory notebook pages were performed under my direct supervision prior to November 5, 1998.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may

jeopardize the validity of the application or any patent issuing thereon.

9/3/02
Date

Eric F. Bernstein, M.D.

[redacted]

Received sample from Jana Eckhard — Store -20°C (Upper Box)

- 2 × 50ml conical tubes - T410 D-161 (10-31-97)
- 2 × 50ml conical tubes - 472-95 D-174 (10-31-97)
- 1 × 15ml conical tube - Alpha-1-Proteinase Inhibitor 6.8 mg/ml 472-78-8082

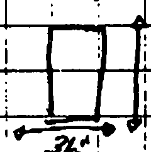
[redacted]

Mix Vitamin D milk with Aquaphor - (from clinic)
Lubriderm - Fragrance Free
Propylene Glycol - [SIGMA] P-1009 (500ml)

Lubriderm : Milk	Aquaphor : Milk	Propylene Glycol : Milk
1:1 ✓	1:1 x	1:1 x
	1:2 x	1:2 x
	1:3 x	1:3 x
	1:4 x	1:4 ?
	1:5 x	1:5 ?
		1:6 ✓

Heating in 65°C bath for 10-15 min helps to dissolve, but Aquaphor tends to solidify. Propylene Glycol did not get into skin very easy until 1:5, 1:6. Lubriderm @ 1:1 with milk = No problem

2 litter of pups - born 4 Nov 97 - Use 2mg/cm² of 1:1 mixture of Lubriderm + D-161 and Lubriderm + D-174



$\frac{3}{4}" = 0.2813\ in^2 = 0.7144\ cm^2$

$2\ mg/cm^2 \Rightarrow \frac{2\ mg}{cm^2} \cdot 0.7144\ cm^2 = 1.4288\ mg = 0.0014\ g$

10 MED UVB = 35m56s			20 MED UVB = 1h11m51s	
1, 2	untreated controls	(cut tail)	1, 2 untreated controls	(cut tail)
3, 4	untreated UVB only	(nothing)	3, 4 untreated UVB only	(nothing)
5 →	UVB + D-161	(FR)	5 → UVB + Lubriderm	(F.L.)
6 →	UVB + D-174	(BL)	6 → UVB + D-161	(F.R.)

[redacted]

L-1-Proteinase 6.8 mg/ml 472-78-8082 (in PBS) 19 c

2 · Cont
2 · UVB (43 sec ≈ 65 mJ/cm²)
2 · 10 μM + UVB
2 · 20 μM + UVB
2 · 40 μM + UVB
2 · 40 μM only

- Incubate for 15 min prior to exposure.
- 43 sec UVB bulbs

MW = ≈ 52,000 g/mol

$6.8\ \frac{mg}{ml} \cdot \frac{1000\ ml}{1\ L} \cdot \frac{1\ g}{1000\ mg} = 6.8\ \frac{g}{L} \cdot \frac{1\ mol}{52.000\ g} = 0.0001307\ \frac{mol}{L}$ (M)

$= 0.1307\ \frac{mmol}{L}$ (mM)

$= 130.7\ \frac{\mu mol}{L}$ (μM)

[redacted] = [redacted]

6.8 mg/ml = 130.7 μM

[2.081 mg/ml = 40 μM]

[1.04055 mg/ml = 20 μM]

[0.52028 mg/ml = 10 μM]

[redacted] dishes (3ml/dish) = [redacted] ml [68.673 mg]

3 dishes (3ml/dish) = 9ml [9mg]

3 dishes (3ml/dish) = 9ml [4.5mg]

[redacted] mg / 6.8 mg/ml = [redacted] 10.0

So, take 10.099 ml of 6.8 mg/ml = 68.673 mg

+ 22.901 ml of PBS = 40 μM conc. (33 ml total volume)

Pre Incubate 4 dishes w/3ml for 12½ min (15 min total) = 12ml
"Shine" 4 dishes w/3ml for 43 sec. = 12 ml
24 ml

9ml Remain @ 40μM conc.
+ Another 9 ml of PBS = 18ml total = 20 μM
Pre Incubate 2 dishes w/3ml = 6ml
"Shine" 2 dishes w/3ml for 43 sec. = 6ml
12ml

51392 -
Anti-Topo... ~54000 Da

$\frac{52{,}000\ g}{mol} \times \frac{1\ mol}{1000\ mmol} \times \frac{1\ mmol}{1000\ \mu mol} \times \frac{1000\ \mu mol}{L} \times \frac{L}{1000\ ml} \times 40\ ml = 2.08\ g$

1) cont
2)
3) UVB (43s)
4)
5 0.01 μM
6 0.1
7 1
8 10
} UV + 43s Incub. 5m.
9 0.01 μM
10 0.1
11 1
12 10
} UV + 43s Incub 15m.

10 μl antib

		Pt.	Tris
1	0.2920	56.8	43.2
2	0.2750	60.4	39.6
3	0.1660	100 μl	0
4	0.1770	93.8	6.2
5	0.2100	79.0	21.0
6	0.2270	73.1	26.9
7	0.2360	70.3	29.7
8	0.2180	76.1	23.9
9	0.2420	68.6	31.4
10	0.1910	86.9	13.1
11	0.2250	73.8	26.2
12	0.2030	81.8	18.2